REMARKS

Preliminarily, Applicants wish to thank the Examiner and Supervisory Examiner Reynolds for the courtesy of granting a personal interview to Applicant John Shannon and his legal representative on January 7, 2004.

Reconsideration and allowance of the above referenced application is respectfully requested.

Claims 23-32 are currently pending in the present application. Claims 24, 25, 27, 30, and 32 have been withdrawn from further consideration.

Double Patenting

The Examiner advised that should Claim 23 be found allowable, Claim 31 would be objected to under 37 CFR §1.75 as being a substantial duplicate thereof. Applicants respectfully disagree with the Examiner's opinion that Claims 23 and 31 are substantial duplicates. Claim 31 includes the additional required step of "cultivating sponges ... in a sponge bed" whereas Claim 23, which does not recite that step, is of somewhat broader scope. In that the two Claims do not have identical steps and define definitions of different scope, Applicants respectfully request that should the Examiner determine Claim 23 to be allowable, the Examiner will not proceed to reject Claim 31 as being double patenting of the subject matter of Claim 23.

Claim Rejections - 35 U.S.C. § 112

Claims 23 26, 28, 29 and 31 are rejected under 35 U.S.C.§ 112, first paragraph, as failing to comply with the enablement requirement. Applicants respectfully traverse the rejection.

The basis for the Examiner's rejection of the claims under 35 U.S.C. § 112, first paragraph is summarized in three parts in the first full paragraph of page 5 in the Official Action

dated October 24, 2003. Those three parts are: 1) a lack of guidance for cultivating bacteria on a sponge, 2) cultivating bacteria in sufficient numbers so as to concentrate the metals or minerals, and 3) a lack of evidence that any symbiotic bacteria of a sponge could concentrate metals or minerals. During the course of the personal Examiner's Interview of May 1, 2003, the telephonic Examiner's Interview of November 4, 2003, and the personal Examiner's Interview of January 7, 2004, referenced above, each part of the basis for the Examiner's rejection was discussed.

Regarding the first part of the basis for the claim rejection, it was concluded that because cultivation of bacteria on a sponge was not an element of the claimed invention it was not necessary to provide an enabling disclosure of how to cultivate bacteria on a sponge. Further, the Applicants, in the present specification at pages 4-5 disclose that the large bacteria population in sponges (up to 40% of the total mass in some species) is enormously varied, hence there is no need to cultivate bacteria in sponges. In addition, the Examiner's Official Action states in the first full paragraph of page 4 that "Wilkinson et al., 1981 (Microb. Ecol., Vol. 7, p. 13-21) indicates that bacterial symbionts are common with many marine sponges and there is a large mixed population of morphologically different bacteria in the mesophyl of marine sponges (e.g. p. 7, 8). Other references cited by the Examiner and discussed in the Examiner's Interview further support the position that the need to cultivate bacteria in sponges is not an issue relevant to the invention as claimed.

Regarding the second part of the basis for the claim rejection, during the course of the telephonic Examiner's Interview of November 4, 2003 and during the most recent Examiner's Interview of January 7, 2004, it was understood that because cultivation of bacteria was not an element of the claimed invention the issue of the number of bacteria cultivate was moot. Further, even if cultivation of the bacteria was an element in the claims, the number of bacteria cultivated would not be an issue of patentability since the amount of bacteria cultivated, the amount of

metal accumulated, and the resulting economic value of the method are not elements of the claims and are therefore irrelevant to enablement of the invention as claimed.

As discussed above, all issues earlier raised in the examination of the present application have been address with the exception of the third part of the basis for the claim rejection of enablement under 35 U.S.C. § 112, first paragraph. In paragraph 5 of the Examiner's Official Action, the Examiner cites a lack of evidence that any symbiotic bacteria of a sponge could concentrate metals or minerals. Responsive to this third part of the basis of the claim rejections, Applicants presented publication evidence that was discussed and made of record by the Examiner in support of the Applicants' position that enablement of the invention as claimed is fully supported by specification. (Gardea-Torresdey et al., Ability of Immobilized Cyanobacteria to Remove Metal Ions From Solution and Demonstration of the presence of Metallothionein Genes in Various Strains, Jn.Haz.Subst. Res., Vol. 1., 2-1 - 2-18 (1998); Carpenter, E.J., Marine Cyanobacterial Symbiosis, Biol. & Envir: Proc. of the Royal Irish Acad., Vol. 102B, No. 1, 15-18 (2002); and Unson, M.D. et al., A Brominated Secondary Metabolite Synthesized by the Cyanobacterial Symbiont of a Marine Sponge and Accumulation of the Crystalline Metabolite in the Sponge Tissue, Marine Biol., 119: 1-11 (1994)). Those references are also provided in a concurrently filed Information Disclosure Statement.

On the basis of the three references cited above and discussed during the Examiner's Interview of January 7, 2004, the Examiner, with the concurrence of Supervisory Patent Examiner Reynolds, conceded that the application was enabling for cyanobacteria, which was an example described in the specification of the present application.

Further discussions regarding the Applicants' position that the disclosure was enabling for the full breadth of the invention as claimed resulted in a decision by the Supervisory Patent Examiner and Examiner that it would be a sufficient standard of proof of bacterial metal

accumulation to show bacteria class that are symbiotic with sponges also possess a gene for metallothionein. The Examiner's Interview concluded with the agreement that the Applicants would submit publication evidence to meet that standard of proof.

In agreement with the above referenced Examiner's Interview, Applicants herewith provide a concurrently filed Information Disclosure Statement listing the requested publication evidence showing example bacterial classes known to be symbiotic with sponges and also known to possess a gene for metallothionein. Each of these evidence publications are briefly discussed in pertinent part below.

Provided are published papers referencing the attributes of four genera of bacteria. Two of the genera are cyanobacteria provided as additional evidence to that which was already provided during the Examiner's Interview of January 7, 2004. Those bacteria are *Synechocystis* and *Oscillatoria*.

The following publications provide evidence that *Synechocystis* and *Oscillatoria* are both known symbionts of sponges and are both known to have a metallothionein gene. See Carpenter et al. (2002) at p. 1 ("Associations [with sponges] occur ... *Synechocystis*, *Oscillatoria* ...") and see Gardea-Torresdey et al. at pp. 2-9, 2-15 ("An important resistance mechanism may be the production of metallothioneins." "Metallothionein genes were shown to be present not only in *Synechococcus* sp. PCC 7942, but in seven other cyanobacterial strains." For *Oscillatoria* see Unson et al. (1994) p. 1 ("The dominant prokaryotic endosymbiont in the mesohyl of the sponge is a filamentous cyanobacterium (*Oscillatoria spongeliae*)" "Virtually all sponges harbor prokaryotic endobionts") See Kelecom at 163 (2002) (See Table X and "Examination of the sponge established that it contains up to 50% of cellular volume of the cyanobacteria symbiont *Oscillatoria spongelliae*..." See Liu et al. (2004) (A metallothionein ... identified and characterized from the cyanobacterium *Oscillatoria brevis*." and see Brooks at 168 (1992) (under

"Specific Examples of Bioaccumulation of Noble Metals", "cyanobacteria (*Phormidium, Oscillatoria,* and *Mastigocladus"*)

The following publications provide evidence that genus Bacillus are known symbionts of sponges and are also known bioaccumulators of metals or minerals. The provided references shows that the genus *Bacillus* accumulates metals without necessarily using the metallothionein mediated pathway. Metal accumulation by any pathway is supportive of the Applicants' claimed invention but it remains that genus *Bacillus* may also possess a metallothionein gene. See Burja et al. at 2, in discussion microbial symbionts of Great Barrier Reef sponges, the genus "*Bacillus*" is included. Also see Kelecom at 163 (2002) (Table X, listing "*Bacillus*" with "sponge" Also see Reith at 337 (2003) (in discussing evidence for a microbial mediated biogeochemical cycle of gold, "*Bacillus subtitlis*" is listed for the microbial induced precipitation of gold. Reith also states, "Au^{31} was selectively absorbed by *Bacillus subtilis* ..." and at p. 338, "... uptake of gold has also been shown for other microorganisms such as *Bacillus cereus*.." Also see Brooks at 169 (1992) (under "Specific Examples of Bioaccumulation of Noble Metals", "The exact location of metal-binding sites in gold-accumulating bacteria has been examined in the case of *Bacillus licheniformis*."). Also see Langley et al. at 489 ((1998) ("Numerous studies have examined the metal ion-cell wall interactions of gram-positive bacteria (particularly members of the genus *Bacillus*...The sites responsible for metal binding in this organism are probably the carboxyl sites with the peptidoglycan, as well as the phosphoryl groups of the teichoic and teichuronic acid second polymers."

The following publications provide evidence that genus *Pseudomonas* are known symbionts of sponges, are known to have a metallothionein gene, and are known bioaccumulators of metals or minerals. See Kelecom at 155 (2002) ("Among the carotenes, a rare C^{50} homologue, okadaxanthine (4), has been isolated from the bacteria *Pseudomonas* sp. strain KK 10206C

obtained from an homogenate of the sponge *Halichondria okadai*." Also *id.* at 163 (Table X, see "*Pseudomonas*" and "sponge") *id.* at 165 ("Two unidentified bacteria, *Pseudomonas* sp and *Alteromonas* sp, have been isolated from *H. okadai* homogenates." See Jayatilake et al. at 293 (1996) ("Microorganisms associated with marine invertebrates are considered to be of particular importance since metabolites previously thought to arise from the invertebrates may be biosynthesized by their endobionts. *Isodictya setifera* Topsent (family *Esperiospsidae*) is a bioactive antarctic sponge from which we have isolated microorganisms for evaluation of their bioactivity. One of the associated bacteria, a strain of *Pseudomonas aeruginosa* (*Psedomonadaceae*), exhibited strong antibacterial activity...)") See Higham et al. at 5 (1986) ("Metallothioneins are a ubiquitous class of proteins and have been isolated from a wide range of animals and several microorganisms including ...cyanobacteria.." "Metallothioneins are implicated in metal homeostasis and detoxification." Also see *id.* at 10 ("The isolation of pseudothioneins adds important information to our knowledge of the occurrence of low molecular weight metal-binding proteins. Although the bacterial proteins resemble metallothioneins, there are several significant differences, especially in their lower cystein content. This implies that amino acids other than Cys are involved in metal binding. The lower Cys content and presence of aromatic amino acids in the pseudothioneins are also characteristics of a metallothionein isolated from cyanobacteria." "*Pseudomonas putida* actively accumulates cadmium ...") Also see Reith at 338 (2003) "...some of these organisms, such as *P. islandicum*, ... are capable of precipitating gold by reducing Au^{3l} to Au(0)..." Also see Malekzadeh et al. at 1 ("*Pseudomonas* MGF-48, a gram-negative, motile, oxidase negative, catalase positive, yellow pigmented bacterium, isolated from electroplating effluent, was found to accumulate heavy metals, especially uranium." "Various microbial species, mainly *Pseudomonas*, have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead, and other metal ions ..."

"We report here the bioaccumulation of several metals by a bacterium, identified as a *Pseudomonas* sp., strain MGF-48, isolated from the effluent of a metal melting factory in the south of Tehran.") *id.* at 3 ("*Pseudomonas* MGF-48 was capable of accumulating several metals (Pb, Cd, Cu, Ag, Ni)..." *id.* at 4 ("*Pseudomonas putida* II-11, ... showing that it accumulated Cu(II), up to 6.5% dry weight..." "*Pseudomonas* MGF-48 appears to accumulate uranium in the cell wall and along the external cell surfaces, as well as internally.") Also see Langley et al. at 1 (1998) ("*Pseudomonas aeruginosa* PAO1...Strain PAO1 (A+b+) and three isogenic LPS mutants ... were studied..." "The results indicated that cells of all the strains caused the precipitation of gold as intracellular, elemental crystals ..." "All four strains bound similar amounts of copper ..." "Strain dps89 caused the precipitation of iron ... on the cell surface, while strain AK1401 nucleated precipitation of lanthanum ... as apiculate, surface-associated crystals." "The gold appeared to precipitate with the cytoplasm of the cells as electron-dense, colloidal aggregates...the aggregates produced diffraction patterns with lattice or d- spacings ..., which are indicative of metallic gold." In this final example, the bacterial species *Pseudomonas aeruginosa* was shown to be both a symbiont and a stellar bioaccumulator.

In sum, the provided references meet the standard of proof set during the January 7, 2004 Examiner's Interview for showing bacteria classes known to be symbionts of sponges and also known to be have a metallothionein gene. These references are therefore submitted for study by the Examiner as evidence that the Applicants invention as claimed meets the enablement requirement of under 35 U.S.C. § 112, first paragraph. From the evidence provided it is clear that in addition to the cyanobacteria example of the specification, which has already been acknowledged during the Examiner's Interview to be fully enabled, the Applicants are also entitled to the breadth of scope of the invention disclosed and claimed in the present application; that is, to bacteria that are harbored in a sponge, have a symbiotic relationship with the sponge,

and are capable of concentrating metals or minerals. Accordingly, the Applicants respectfully request that the Examiner withdraw the rejection of Claims 23, 26, 28, 29, and 31 as being not enabled under 35 U.S.C. § 112, first paragraph.

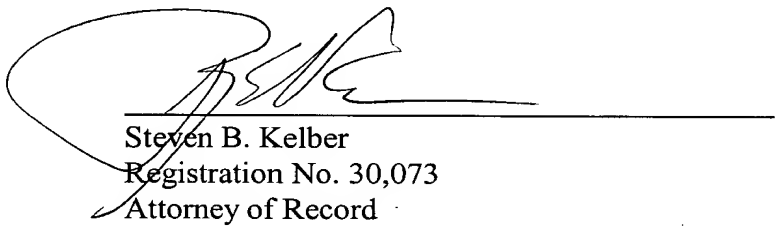
CONCLUSION

In light of the above, Applicants believe that this application is now in condition for allowance and therefore requests favorable consideration.

If any points remain in issue which the Examiner feels may be best resolved through a personal or telephonic interview, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

PIPER RUDNICK LLP



Steven B. Kelber
Registration No. 30,073
Attorney of Record

Perry E. Van Over
Registration No. 42,197

1200 Nineteenth Street, N.W.
Washington, D.C. 20036-2412
Telephone No. (202) 861-3900
Facsimile No. (202) 223-2085